one has been studied. Indium was found to be extracted with chloroform solutions of dithizone under the same conditions as the lead, tin, thallium, and bismuth group. Tentative procedures have been presented for the separation of indium from various metals and its determination by dithizone methods.

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PLANT PATHOLOGY.—Bacillus megaterium *de Bary from the interior of healthy potato tubers.*[1]

Benjamin Franklin Lutman and Harry E. Wheeler, Department of Botany and Plant Pathology, Louisiana State University. (Communicated by Nathan R. Smith.)

For several years the writers have been attempting to grow microorganisms from the filamentous plant intercellular inclusions which had been described by Lutman (*6*, *7*). Various methods were used to induce them to leave their intercellular habitat and grow in another medium, but without success. Although a few actinomycetes were occasionally obtained, no assurance could be given that they were not accidentally introduced in the transfer of material from tubers to medium.

The technique that was used in the following work was neither new nor complicated. Burbank Russet tubers, grown in Idaho, were used for much of this work because they were available in the market and their long shape made them easy to break. At the time most of these trials were made these mature tubers showed sprouts, indicating that the rest period had been passed.

Clean, selected tubers were disinfected for 2 hours in 0.5 percent formaldehyde solution. They were dried and then cut on one side so that they could be broken readily. Disks of tuber tissue were removed with a sterile cork borer and a scalpel. Usually three or four of these circular disks (1 cm across and 1–2 mm thick) were taken from the broken surfaces and removed to bottles of sterile water. After washing they were placed in a small, sterile porcelain mortar, ground to a fine paste, and transferred to another similar flask of sterile water. The material from these flasks was plated out on nutrient agar to which had been added 2 percent dextrose and 1 percent yeast extract.

The broken tubers were placed between layers of sterile filter paper in a glass dish for five or six days, and then they were again used for samples. In this time the cut surfaces had developed a new cork layer from a cork cambium. The walls of the new cork cambium were filled with strands of hyphae, indicating that the microorganism had renewed its activity after being dormant in the tuber. The broken surfaces were washed off in 95 percent alcohol and the adhering alcohol burned off. Tissue disks, removed as just described, were broken up in the mortar to a fine paste and diluted 1 to 10,000 before plating.

The number of organisms obtained in the 3 operations varied widely with the tubers used. In one set of trials were 100 colonies per disk from the wash water, 700 colonies after the disk had been broken into fragments, and 33,000 from the disks taken from the regenerated skin. In another trial the numbers were 300 from a washed disk, 1,200 from a ground-up one, and 48,000 from a disk from a regenerated skin layer.

[1] Received August 5, 1948.

The organisms obtained, however, were not actinomycetes, but a large, curved-rod bacillus which was easily identified as the highly pleomorphic de Bary organism, *Bacillus megaterium*.[2] Occasionally, colonies of other organisms appeared on the plates, but the predominant form was always this bacillus.

For a long time these organisms were regarded as intruders from the soil and were disregarded. But, since they were so frequent, even with all precautions taken against the introduction of soil bacteria, a more careful examination was made of the colonies and of the individuals composing them. For this purpose the colonies that resulted from plating out the tissues were used, their age usually being 24 to 72 hours.

Colony margin.—The filaments protruding from the margins of the colony frequently showed branching, although in some colonies the individuals were pressed together in parallel, concentric circles to form a smooth margin. The most striking variation from an ordinary bacterial colony was the long filaments that would break away from the colony margin to push out across the agar to some distance, where they would bud off a new, small daughter colony. Rettger and Gillespie (*9*) gave the appropriate name of "runners" to these long nonseptate and unfragmented hyphae and offered the explanation that their appearance was stimulated by a lack of oxygen in the parent colony.

Reproduction and unusual individuals.—The unusually large size of the organisms (1.2 to 1.7 microns in width and averaging 5 to 10 microns in length) gave an opportunity to observe the details of cell reproduction by budding. Smith, Gordon, and Clark (*10*) in their monograph of this group state that *B. megaterium* produces apical and side buds. This description of bud formation is shorter and clearer than that of de Bary (*2*) who goes into considerable detail in the growth of the buds and the curvature of the rods: "The rods divide by the formation of a transverse septum into two members, the transverse septa are extremely delicate when young. When two sister rods begin to separate transversely from one another, the curvature usually becomes more pronounced at the extremities where division takes place, and the ends of the rods become slightly oblique to one another and overlap each other a little, or one thrusts itself laterally past the other, like the short commencement of a so-called false branch in Syctonema and similar genera of the Nostcaceae." His figure, however, shows clearly side buds, although all buds originate near cell apices.

Budding would automatically deny the name of the group to which it had been assigned, the Schizomycetes (fission-fungi). As a result of the buds and continued apical growth, true branching is common in the cells of these colonies derived directly from potato tissue. On replating these colonies, however, the branches or buds were shorter, so that the curved rods usually considered typical for this species now predominated. Rettger and Gillespie (*9*) had noted that these so-called "abnormalities" were always more frequent near the colony margins where oxygen supply was more abundant. At the centers and in the depths of the colonies fragmentation into short curved rods introduced the "normal," i.e., the laboratory type, of the species. The long filaments, branching cells, etc. were not permanent, but would resume their normal form if returned to any of the standard media (*4*).

Spore formation and sheath.—Occasional long filaments may be seen in the colonies. They fragment by the introduction of biconvex vacuoles. The protoplasm retreats and the empty space enlarges and becomes biconcave. This fragmentation is marked near the end of a filament where the protoplasm frequently fragments in a short branch into three or four capsule-shaped spores enclosed in a clear sheath. These short rows of spores are usually curved as are those of the actinomycetes. These spores would be the conidia of these latter filamentous species and are to be distinguished from the endospores described in some strains of *B. megaterium* but which are absent in other strains, and may be lost occasionally from those having them (*5*).

[2] The authors will use the original de Bary spelling.

No endospores were observed on these strains when grown on dextrose agar.[3]

Rettger and Gillespie (*8*) noted so-called "empty sausage" skins (sheaths empty of protoplasm) but did not consider them of special importance, presenting their observations "as a matter of general interest, rather than as evidence of the occurrence of a highly specialized cell membrane or envelope in bacteria." They did not call attention to the fact that the actinomycetes have such an envelope or sheath, even if other microorganisms do not. This envelope or skin has been investigated by a number of electron microscope workers. Dubin and Sharp (*3*) arrived at the conclusion that "electron micrographs indicate plainly the presence of two structures constituting the bacterial cell, an inner dense substance and outer less dense substance. The outer substance of the bacterial cell is invisible in light micrographs." To the latter statement, the authors would in part disagree, since this sheath may often be seen between the fragments of protoplasm, especially after dense staining.

Germination of the intercellular strands.—The best proof of the origin of the colonies from the intercellular filaments would be to grow them from between the cells out into a culture medium. In order to demonstrate this point a somewhat different technique was used.

Some of the ground-up regenerated cork layer in a 100-cc water suspension was pipetted on large (24 by 50 mm) cover glasses, where it was allowed to air dry. A thin layer of the nutrient agar used in the plates was then spread over these dried tissue fragments. After it had hardened the cover glass was inverted over a slide, the ends of the cover glass being suspended by fine glass rods. The slide was then placed in a damp chamber.

In a warm room germination would begin in about two to three hours. Probably more than 90 percent of the germinations would have no connection with bits of tissue. They seemed to originate in fragments of mycelium loosened from their intercellular attachments by the pounding in the mortar. No indication of remains of a spore could be seen even in the early 1- and 2-celled stages. They were bits of mycelium free from all tissue connections. This fact would indicate that the mycelium is not deeply imbedded in the pectin of the intercellular region.

An occasional filament can be traced back into strands inside the tuber tissue. Such a filament will be seen to break up in the culture medium into the rods of *B. megaterium*. In the two figures shown, the dried tissue had been covered with agar at 11:30 A.M. When examined and photographed at 2:30 P.M. the filament was projecting into the medium and had thrown off a long bud, which lay parallel to it. The growth was rapid during the first half hour, but a series of five exposures were made of which only one, that at 4:30 is reproduced. At this time the true point of origin of the cluster of bacilli could be determined only by referring back to the earlier stage.

A careful study of such filaments germinating from tissue showed that in every case the part in the tissue lay close to the margin and that all connection with the hyphae of the cells had been broken. In no instance was a germination observed from the end of a hyphae extending unbroken back into the tissue.

Occurrence in other parts of the potato plant and in other plants.—No extended efforts were made to isolate *B. megaterium* from other parts of the potato or from other plants. It was noted that, with the same technique used, this organism was common in disinfected sweet potatoes and also in potato roots after they had been washed and dipped in 95 percent alcohol from 10 to 15 seconds before crushing them up in the mortar. The same is true of garden carrots.

Since the Burbank Russet potatoes were mature and had been in storage for at least four months, the same technic was used on small Bliss' Triumph tubers taken fresh from the soil and larger tubers of the same variety grown in Florida. The same organisms appeared as when the storage tubers had been used.

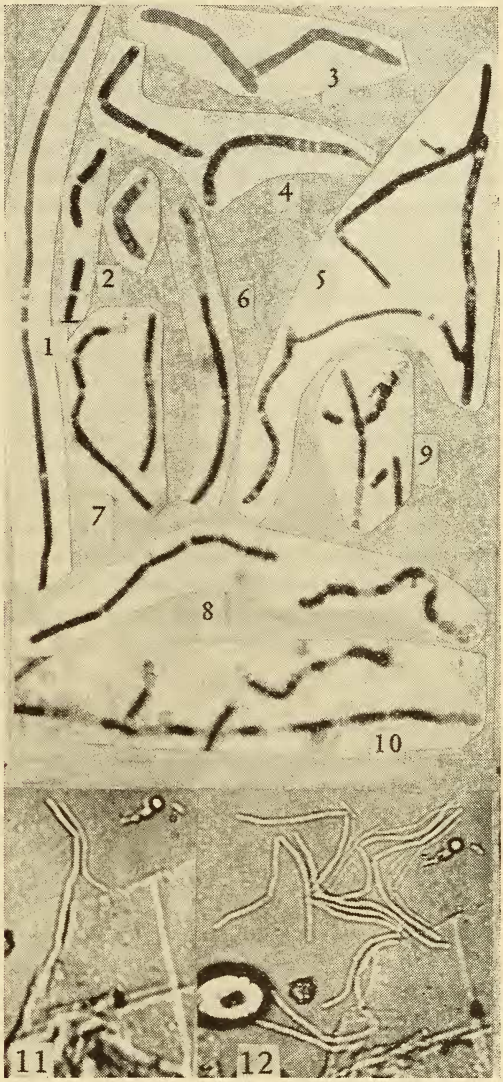
[3] The writers wish to thank Dr. Nathan R. Smith for the additional information that the three isolations sent to him after this work was done readily produced spores on the ordinary beef agar.

Sheath on the hyphae in the tubers.—No note was taken of the occurrence of a sheath on the filaments stained in the intercellular regions of the potato tuber by Lutman (*7*), but such a sheath shows distinctly in many of the photographs, especially those of sections of mature tubers. In one of the illustrations of these filaments in the turnip, the tube is shown cut through in such a manner that the organism inside it was missed and the tube in part seemed empty. As shown in the same paper the enclosed filaments were also Gram-positive, although a special timing and technique had to be used to demonstrate them.

Tissue cultures with sterile material.—The interior of healthy plants was formerly held to be free from bacteria and sterile. Tissue cultures have been made of many organs without any evidence of bacterial growths on the bits of tissue. The reason for this apparent freedom from bacteria is due, so far as *B. megaterium* is concerned at any rate, to its marked aerobic habit. It will not grow in a stab in a solid culture medium to a depth of much more than two millimeters. In tissue-culture technique, the bits of tissue are covered with the nutrient fluid to a depth sufficient to stifle the growth of this highly aerobic species. If growth should start, the colonies would be so small owing to lack of oxygen that they have been overlooked.

Systematic position of the organism.—The question arises at once in the mind of any systematist of microorganisms of the naming and grouping of the pleomorphic species which lives part of its life as a branching mycelium inside plants and breaks up into short, motile bacilli in culture media.

In the sixth edition of Bergey's *Manual of determinative bacteriology* (*1*), Order II. *Actinomycetales* Buchanan has as family I. *Mycobacteriaceae* Chester, a single genus: I. *Mycobacterium* Lehmann and Neumann. This group contains many important organisms such as those associated with tuberculosis and leprosy. These organisms are also filamentous at times but break up readily into nonmotile rods. *Bacillus megaterium* would not fit into such a genus since the organisms are typically motile. It can not be regarded as a true Actinomyces, although it has a sheath and the resting spores are similar to those of the latter group. No member of the Actinomyces



Figs. 1–12.—Pleomorphic forms assumed by *Bacillus megaterium* in culture media, aqueous crystal violet stain: 1, "Runner" type with vacuoles; 2, types of short bacilli; 3, budding, and 4, bud almost separated; 5, extreme branching with variation in size of branches; 6, end of filament with empty "sausage" skin; 7–10, resting spores formed by fragmentation, spores still enclosed or connected by clear-walled sheath; 11, filament arising from a bit of potato tuber tissue with a parallel branch or bud at right, taken at 2:30 p.m.; 12, same, at 4:30 p.m. (Figs. 1 and 5–9 magnified 1,100 times, Figs. 2–4 magnified 1,350 times; 11 and 12, 400 times.)

is known, however, to have a motile stage or endospores. The intermediate position of this microorganism is clearer than its disposition in any present classification.

The middle lamellae.—To the botanist the fundamental contribution of these observations is that the denser material between plant cell walls is not a chemical (calcium pectate), as suggested by Mangin who discovered these bodies, but living microorganisms that may be grown in culture media outside the plant. Further, the old conception that the interior of plants is sterile is not tenable. The role which these microorganisms play in the physiology of the higher plants will have to be determined by future experiments, but the abundance of the filaments in enlarged roots (carrots, beets, turnips) and tubers (potato, sweet-potato, and Jerusalem artichoke) suggests the formation of some type of growth-stimulating substances. It may be pointed out that the invasion of the cork cambium of young potato tubers by a similar microorganism stimulates the cork cells to produce hypertrophied tissue known as common or corky scab.

ADDENDUM

The day following the receipt of the manuscript for transmittal to the editors of the JOURNAL, word was received of the fatal illness of the senior author of this paper. In the meantime it has come to my attention that G. B. Sanford recently published a paper in *Scientific Agriculture*, vol. 28, pp. 23–25, 1948, entitled *The occurrence of bacteria in normal potato plants and legumes*. In addition, I am informed that a manuscript by Tervet and Hollis along the same line has been accepted for publication and will shortly appear in *Phytopathology*. These and former papers seem to leave little doubt that healthy plant tissues may contain microorganisms. The frequency of their occurrence and their function still remain to be discovered. —NATHAN R. SMITH.

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ZOOLOGY.—Hesperochernes thomomysi, *a new species of chernetid pseudoscorpion from California.*[1] C. CLAYTON HOFF, University of New Mexico. (Communicated by EDWARD A. CHAPIN.)

Pseudoscorpions are common in the nests of burrowing rodents. The species found in rodent nests have received relatively little attention, however, perhaps as a result of difficulties encountered in making species determinations in the groups to which most of these forms belong. In the present paper, a new species of the genus *Hesperochernes* is described from the nest of *Thomomys monticola* from California, the description being based on material submitted by Dr. Edward A. Chapin, of the United States National Museum. The type specimens mounted on microscope slides are deposited in the National Museum. As a result of our very inadequate knowledge of chernetid

[1] Received June 7, 1948.